

PATENT
ATTORNEY DOCKET NO. 50154/002002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Hubbell et al. Confirmation No.: 5903
Serial No.: 09/496,231 Art Unit: 1651
Filed: February 1, 2000 Examiner: L.B. Lankford
Customer No.: 21559

Title: BIOMATERIALS FORMED BY NUCLEOPHILIC ADDITION
REACTION TO CONJUGATED UNSATURATED GROUPS

DECLARATION UNDER 37 C.F.R. § 1.131 OF PRIOR INVENTION

Under 37 C.F.R. § 1.131, we declare:

1. We are the inventors of the subject matter that is described and claimed in the above-captioned patent application.
2. The enclosed Exhibit is a copy of pages from the laboratory notebook of inventor Alison Pratt, which show that we had reduced to practice the generic invention of the relevant claims prior to August 20, 1998. In particular, these pages show conception of the reaction of multi-cysteine peptides, containing multiple nucleophilic groups, with poly(ethylene glycol) (PEG) acrylates and vinyl-sulfone PEG, containing multiple conjugated unsaturated groups. In addition, these pages report experimental results showing the formation of a gel from the reaction of a polythiol, trimethylolpropane tris(3-mercaptopropionate), with PEG acrylate. The work documented in the Exhibit was performed in Switzerland after January 1, 1996 (but before August 20, 1998).

3. All statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

16 April 07

Date

Date

Date

Date

Date

Date

Date


Jeffrey A. Hubbell

Donald Elbert

Matthias Lutolf

Alison Pratt

Ronald Schoenmakers

Nicola Tirelli

Brent Vernon

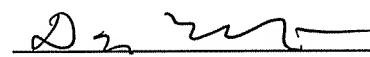
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Date

April 16, 2007

Date

Jeffrey A. Hubbell



Donald Elbert

Date

Matthias Lutolf

Date

Alison Pratt

Date

Ronald Schoenmakers

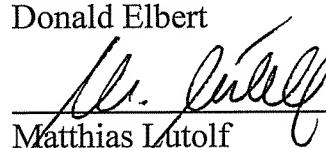
Date

Nicola Tirelli

Date

Brent Vernon

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Date	Jeffrey A. Hubbell
Date	Donald Elbert
<u>25.4. 1973</u>	 Matthias Lutolf
Date	Alison Pratt
Date	Ronald Schoenmakers
Date	Nicola Tirelli
Date	Brent Vernon

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Date

Jeffrey A. Hubbell

Date

Donald Elbert

Date

24 April 2007
Date

Matthias Lutolf


Alison Pratt

Date

Ronald Schoenmakers

Date

Nicola Tirelli

Date

Brent Vernon

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Date

Jeffrey A. Hubbell

Date

Donald Elbert

Date

Matthias Lutolf

Date

Alison Pratt

Date

Ronald Schoenmakers

24.04.2007
Date

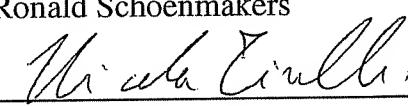


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Date	Jeffrey A. Hubbell
Date	Donald Elbert
Date	Matthias Lutolf
Date	Alison Pratt
Date	Ronald Schoenmakers
25/4/07	
Date	Nicola Tirelli
Date	Brent Vernon

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Date

Brent Vernon



4/17/07

EXHIBIT

(DATES REDACTED)

Gel formation step w/ poly sulfhydryls + acrylates

Goal: to use multi-cysteine peptides w/ enzyme-sensitive sequences to form peg hydrogels in cell-friendly conditions

i. Lys - plasmid site - Cys - plasmid site - Cys
reacted w/ $\xrightarrow{\text{PEG}}$ instead of
w/ the more expensive vinyl sulfone PEG

There is enough literature on vinyl sulfone + S-H reactivity, and there is lots of info on photo-polymerization of thiols + enols; however, I have not seen much / anything w/ photo-polymerization

Gel formation of 8kDA poly(urethane + peg DA)

Poly(urethane (tri-nitrile))

trimethylolpropane triis(β-mercaptopropionate)

cas #: 33007-83-9

Aldrich

(HS-CH₂-CH₂-COOC₂H₅)₃C-C≡N

mw = 398.56 d = 1.21

TMP

acrylate: 8kDA peg (88-107% acylation, page 10+)

conditions:

per Natalie's recommendations from her
work w/ vinyl-sulfone peg and CPS or CPT
containing geopolymers

she suggested concentrations of 5-10 mM thiol
3x (15-30 mM)

she gets the peg to go @ pH 7.4, at 30°C,
hydrogen is faster

Buffer: 50 mM NaBicarb pH 8.4 (NaHCO₃)

co-solvent (b/c TMP² not miscible w/water) : acetone/tri
acetone/tri / water = 1/4

8kDA: 10% solution 50 mg. in 500μl solvent + co-solvent

peg dissolved; then TMP² added + mixed via vortex

tmp² not perfectly miscible still - appears as
suspension of small droplets

let stand for ~30 min. some gelled

in ~60 min. all gelled by appearance